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The goal of the research supported by this grant is to determine the role that biosurfactants and synthetic surfactants play in enhancing the bioavailability of sorbed or immiscible-phase polycyclic aromatic hydrocarbons (PAHs) in particulate media. Increased bioavailability is assessed in terms of increased PAH-degrader population densities (nah gene frequencies) and their activities including the rate and/or extent of biodegradation and degradative gene expression as measured by bioluminescence response and mRNA levels. To achieve the proposed goal, bacterial strains containing specific degradative genes and bioluminescent reporter systems are being used to monitor the effectiveness of surfactants for enhancing the biodegradation of aromatic hydrocarbon contaminants in envrionmental simulations. These genetic marker systems allow for the quantitation of degradative gene frequency and activity. Construction of an improved bioluminescent reporter strain for PAH degradation is currently underway. This approach involves incorporation of a transposon containing the lower naphthalene pathway promoter fused to the lux genes (nah-lux) into the bacterial chromosome resulting in a stable gene fusion present as a single copy per cell.

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Contract Number F49620-92-J-0333
Second Annual Technical Report (June 1, 1993 - May 31, 1994)
(FY91 AASERT) Molecular Probes and Bioluminescent Reporters in Ecological Optimization of Biodegradation

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The goal of the research supported by this grant is to determine the role that biosurfactants and synthetic surfactants play in enhancing the bioavailability of sorbed or immiscible-phase polycyclic aromatic hydrocarbons (PAHs) in particulate media. Increased bioavailability is assessed in terms of increased PAH-degrader population densities (*nah* gene frequencies) and their activities including the rate and/or extent of biodegradation and degradative gene expression as measured by bioluminescence response and mRNA levels.

To achieve the proposed goal, bacterial strains containing specific degradative genes and bioluminescent reporter systems are being used to monitor the effectiveness of surfactants for enhancing the biodegradation of aromatic hydrocarbon contaminants in environmental simulations. These genetic marker systems allow for the quantitation of degradative gene frequency and activity. Construction of an improved bioluminescent reporter strain for PAH degradation is currently underway. This approach involves incorporation of a transposon containing the lower naphthalene pathway promoter fused to the *lux* genes (*nah-lux*) into the bacterial chromosome resulting in a stable gene fusion present as a single copy per cell. The strain lacking the *nah*-type plasmid will be used as a control strain to account for potential light production not related to degradative gene expression. By monitoring the light response of the reporter and control strains, definitive results concerning increased PAH bioavailability due to the surfactant treatments will be obtained. In addition, potential toxic effects of the surfactants can be assessed as well.

Currently, a strain containing a plasmid-encoded *tod-lux* gene fusion has been used. This strain produces light in the presence of the inducer, toluene, while the toluene dioxygenase can catabolize TCE as well as toluene. Preliminary studies have shown that this strain will respond to as little as 0.1 ppm toluene and that the light response is proportional to concentration up to 10 ppm toluene. This strain will also emit light in response to JP4 jet fuel components dissolved in water. The relationship between the toluene concentration in these samples and the light response are being determined. The use of this strain has allowed for the optimization of the bioluminescent assays for assessing bioavailability.

The strain containing the *nah-lux* gene fusion (along with the control strain) will be used in batch and continuous-flow (column) systems. These systems will be composed of subsurface soil or aquifer material contaminated with PAH mixtures or immiscible phases containing PAHs (JP4 jet fuel) to evaluate the effect of biosurfactants on PAH desorption and /or dissolution and biodegradation. Corollary studies will be performed with synthetic surfactants to determine whether biosurfactants or synthetic surfactants are more effective in enhancing PAH bioavailability and biodegradation.

Preliminary experiments using a purified rhamnolipid surfactant, R1, produced by *Pseudomonas aeruginosa* PRP652 showed that surfactant concentrations above the critical micelle concentration (CMC) resulted in approximately 12 times more phenanthrene desorbed from soils relative to treatments without surfactant. Likewise, R1 concentrations above the CMC significantly increased the removal of phenanthrene and anthracene from manufactured gas plant soils. Subsequent mineralization experiments have shown that the micellarized phenanthrene is bioavailable. The gene probe technology and bioluminescence monitoring will be applied to these surfactant-soil systems to better evaluate whether surfactants enhance the bioavailability of sorbed or immiscible-phase PAHs.

Academic Prog

The student supported on Contract No. F49620-92-J-0333, Staci R. Kehrmeyer has made satisfactory academic progress towards her Ph.D. requirements. The following courses were taken with the appropriate letter grades indicated below:

Academic Year 1992-1993
Biochemistry 511-Advanced Concepts in Protein Structure, Protein Function and Intermediary Metabolism (A)
Biochemistry 512-Advanced Molecular Biology (A)
Microbiology 470-Microbial Ecology (A)
Microbiology 670-Advanced Topics in Environmental Microbiology (A)
Mathematics 405-Models in Biology (A)
Chemistry 431-Radioactive Tracer Techniques (A)

Academic Year 1993-1994
Geology 485-Principles in Geohydrology (A)
Microbiology 670-Advanced Topics in Environmental Microbiology (B+)